Prevalence of 16S rRNA methylase genes in Gram negative isolates in Athens Metropolitan area in a six month period

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Background: Methylation of 16S rRNA is an important mechanism of high level aminoglycoside resistance among Gram-negative pathogens. The aim of this study was to investigate the prevalence of 16S rRNA methylase genes in consecutively collected Gram-negative isolates in the first semester of 2016 in Athens, Greece.

Material/methods: Single-patient, Gram-negative clinical isolates resistant to both amikacin and gentamicin (n=174), were consecutively collected during a six month period (Jan-June 2016) in five tertiary-care hospitals in Athens. All isolates were sent to a central laboratory for MIC determination to
amikacin, gentamicin, tobramycin [collectively referred to as 4, 6-disubstituted aminoglycosides, (4, 6-A)], apramycin and neomycin with the broth dilution technique. Isolates with MICs ≥256 mg/L to 4, 6-A were examined for the presence of 16S rRNA methylase (RMT) genes (armA, rmtB, rmtC, rmtA, rmtD and npmA) by PCR. Carbapenemase production was confirmed by PCR in all RMT-positive isolates.

**Results:** *A. baumannii, P. stuartii, K. pneumoniae, P. aeruginosa and E. coli* resistant to amikacin and gentamicin were isolated at participating institutions at a rate of 67.8%, 55.1%, 10.3%, 10.1% and 0.4%, respectively. One hundred and eight *A. baumannii* isolates of 113 tested (95.6%), were positive for armA. The vast majority of armA-bearing *A. baumannii* strains were OXA-23 producers (97.2%) while three isolates (all from the same hospital) were OXA-24 producers (2.8%). All *P. stuartii* (n=14) isolates were VIM-producers and harboured rmtB. Eleven of the 29 *K. pneumoniae* isolates (37.9%) harboured rmtB (n=10) or armA (n=1). All rmtB-positive isolates were KPC-producers, while the armA-positive isolate was an OXA-48-producer. None of the 17 *P. aeruginosa*, isolates was positive for an RMT gene although some were highly resistant to 4,6-A tested (MICs >=512 mg/L). One *E. coli* isolate harboured rmtB and was a KPC-producer. The overall prevalence of armA-positive *A. baumannii* isolates was 64.8% and of rmtB-positive *P. stuartii* isolates was 55.1%. *K. pneumoniae* harboring rmtB or armA was isolated in low prevalence of 3.9% while only one rmtB-positive *E. coli* isolate was found in a total of 576 isolates (0.2%).

**Conclusions:** RMT production is an emerging mechanism of resistance, capable to compromise the clinical efficacy of aminoglycosides. High prevalence of RMTs was observed among *A. baumannii* and *P. stuartii* strains isolated in participating hospitals in Athens. Since the previous surveillance study by our group in 2009 (Galani et al. CMI 2012), the prevalence of RMTs in *K. pneumoniae* has increased from 0.4 to 3.9% while no RMTs have been found in *P. aeruginosa*. All RMT-positive isolates were carbapenemase producers.